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# Influence of Angiotensin Receptor Blocker (Candesartan) and Angiotensin-Converting-Enzyme Inhibitor (Enalapril) Combined with Glimepiride on Glycated Hemoglobin in Non-Insulin Dependent Diabetic Rats (NIDDM)

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors WAD, FDEH, AK and AAB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors WAD, RAS, EM and AAB managed the analyses of the study. Authors ZZ, MI and WAD managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** Antihypertensive agents like Angiotensin-Converting Enzyme Inhibitors (ACEIs) and Angiotensin-Converting Enzyme Blockers (ARBs) are commonly indicated for patients with both hypertension and diabetes. However, the effect of these agents on blood sugar level or glycated hemoglobin (HbA1c) is still controversial. This study aims at investigating the short, and long term

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effects of ACEIs and ARBs on blood sugar level and HbA1c of a group of streptozocin (STZ)-induced NIDDM rats when given in combination with Glimepiride (antidiabetic drug from Sulfonylureas group).

**Methods:** Diabetes mellitus (DM) was induced in 100 Wistar albino adult male and female laboratory rats above 8 weeks old, and weigh between 250-300 gm by the administration of Streptozocin 75% α-anomer. Two weeks later, the 100 rats were then randomized into four groups (25 rats each). Group one was the untreated control group (received placebo only), while other groups (II, III, and IV) were treated by Glimepiride only, Glimepiride plus ARB (Candesartan), and Glimepiride plus ACEI (Enalapril) respectively. HbA1C levels were measured at baseline (pre-test/directly after randomization) to ensure that there was no significant difference between study groups at the baseline, post-test (after two weeks), and delayed-post-test (12 weeks after randomization/ 10 weeks after post-test) to measure short and long-term changes in the study groups.

**Results:** There was no significant difference (p-values >0.05) between the four groups (groups I, II, III, and IV) in the HbA1C mean level at the beginning of this study (two-weeks after randomization and injection of STZ) (mean = 7.62 ±SD = 0.41, 7.72 ±SD = 0.48, 7.66 ±SD = 0.47, and 7.52 ±SD = 0.51respectively). However, two weeks later, treated groups (groups II, III, and IV) showed moderate reduction of HbA1C mean level compared to the untreated (placebo) group I, that was significant in groups III, and IV, and insignificant in group II (mean =7.43±SD 0.54, 6.97±SD 0.33, 6.72±SD 0.26, and 7.71 ±SD 0.44 respectively). Furthermore, treated groups (groups II, III, and IV) showed significant dramatic reduction of HbA1C mean level when compared to the untreated group (group I) (mean = 6.22 ±SD 0.51, 5.24 ±SD 0.62, 5.22 ±SD 0.13, and 7.62 ±SD 0.42 respectively).Overall, treated groups showed significantly lower HbA1C level than placebo groups. Moreover, Glimepiride + Enalapril combination showed a stronger hypoglycemic effect than the Glimepiride + Candesartan combination at post, and post-delayed tests, however, these differences were not significant.

**Conclusion:** The addition of either ACEIs like Enalapril, or ARBs like Candesartan to Sulfonylureas like Glimepiride to in NIDDM patients will synergize its anti-diabetic effect in NIDDM subjects, and might increase the possibility of hypoglycemia. Caution and/or dose adjustment should be considered upon using these agents together in patients with hypertension along with diabetes.

*Keywords: Candesartan; Enalapril; dependent diabetic; HbA1c; glimepiride.*

## ABBREVIATIONS

*AT : Angiotensin receptor*
*BSA : Bovine serum albumin*
*HTN : Hypertension*
*ACEI : Angiotensin converting enzyme inhibitors*
*ARBs : Angiotensin II receptor blockers*
*CKD : Chronic kidney disease*
*CVD : Cardiovascular disease*
*DM : Diabetes mellitus*
*EDTAE : Ethylene diamine tetra acetic acid*
*F.B.S : Fasting blood sugar*
*FDA : Food and drug administration*
*HbA1c : Glycated hemoglobin A1c*
*NIDDM : Non-insulin dependent diabetes mellitus*
*PBS : Phosphate buffered saline*
*SUR : Sulfonylurea receptor*
*PPARγ : Peroxisome proliferator activated receptors gamma*
*I.P : Intraperitoneal*
*STZ : Streptozocin*

## 1. INTRODUCTION

Hypertension (HTN) is an important global health challenge because of its high prevalence and resulting cardiovascular (CVD), and Chronic Kidney Disease (CKD) [1,2]. Additionally, HTN is the leading preventable risk factor for premature death and disability worldwide [2].

Candesartan is an Angiotensin Receptor Blocker (ARB) that is mainly used for the treatment of high blood pressure and congestive heart failure [3]. Candesartan cilexetil is a prodrug that is converted to its active metabolite Candesartan during its absorption (Fig. 1) [4,5].

Angiotensin II acts as a vasoconstrictor. In addition to causing direct vasoconstriction, angiotensin II also stimulates the release of aldosterone [6,7]. Once aldosterone is released, sodium as well as water is reabsorbed. The end result is an elevation in blood pressure.

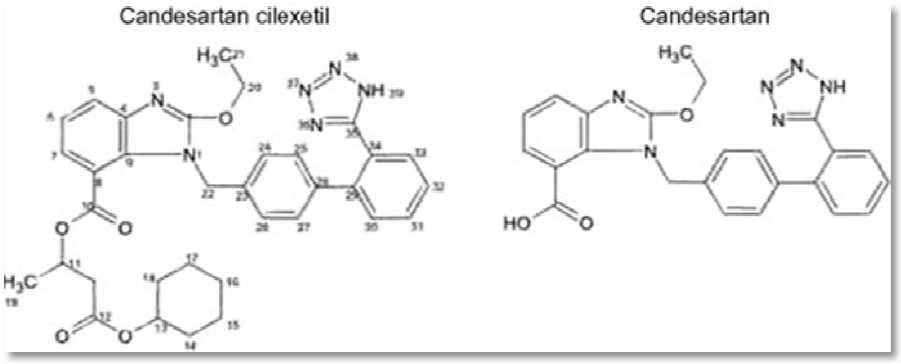


**Fig. 1. Structural formulae of Candesartan and Candesartan cilexetil**

Candesartan binds to the AT1 angiotensin II receptor. This binding prevents angiotensin II from binding to the receptor thereby blocking the vasoconstriction and the aldosterone secreting effects of angiotensin II [5,6].

Enalapril is an Angiotensin-Converting Enzyme Inhibitor (ACEI) that is used to treat high blood pressure, diabetic kidney disease, and heart failure [7]. Enalapril is a prodrug that is converted to active metabolite Enalaprilat (Fig. 2) [7,8].

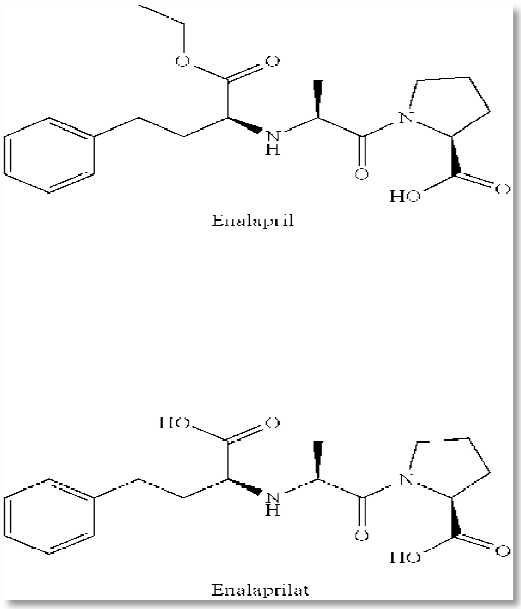


**Fig. 2. Structural formulae of Enalapril and Enalaprilat**

Angiotensin I is converted to angiotensin II by an angiotensin-converting enzyme (ACE). Angiotensin II constricts blood vessels, increasing blood pressure. Enalaprilat, the active metabolite of Enalapril, inhibits ACE. Inhibition of ACE decreases levels of angiotensin II, leading to less vasoconstriction and decreased blood pressure [7,8,9].

Diabetes mellitus (DM), commonly known as diabetes, is a group of metabolic disorders characterized by high blood sugar levels over a prolonged period [9]. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger [9]. Diabetes occurs asa result to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produced [10].

Glimepiride (Fig. 3) is the newest second-generation sulfonylurea and is sometimes classified as a third-generation sulfonylurea because it has larger substitutions than other second-generation sulfonylureas. FDA approved glimepiride in 1995 for the treatment of type 2 Diabetes Mellitus (DM) as monotherapy as well as in combination with metformin or insulin [11].

Glimepiride acts as an insulin secretagogue [12]. Strong evidence is available in the literature for the multiple molecular targets/mechanisms for the blood glucose-lowering effect of glimepiride which operates at both pancreatic ß cells and extra-pancreatic cells [13]. Interaction with the sulfonylurea receptor, SUR, at the ß-cell plasma membrane triggers insulin release [13]. Interaction with lipid rafts, at the plasma membrane of adipose and muscle cells induces the insulin-mimetic activity via the activation of a glycosylphosphatidylinositol-specific phospholipase, redistribution of signaling components and positive cross-talk downstream to the insulin signaling cascade [14]. Interference with additional molecular mechanisms in extra-pancreatic cells (e.g. regulation of adipocytokine

release from and differentiation of adipocytes) relying on or independent of SUR and Dig's contributes to the insulin-sensitizing activity of glimepiride. Glimepiride illustrates a more favorable blood glucose-lowering profile and the lower risk for weight gain, hypoglycemic incidences and CV side effects than glibenclamide due to differences in the engaged targets and or mechanisms [13].

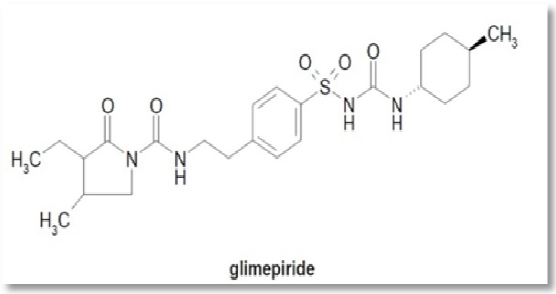


**Fig. 3. Structural formulae of Glimepiride**

Glycated Hemoglobin (HbA1C) is a form of hemoglobin that is covalently bound to glucose. It is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose [14]. It is measured primarily to identify the three-month average plasma glucose concentration and thus can be used as a diagnostic test for diabetes and as assessment test for glycemic control in people with diabetes [15]. The test is limited to a three-month average because the lifespan of a red blood cell is four months (120 days) [14]. The Diagnostic Standard for HbA1c in Diabetes is HbA1C <5.7% as Normal, HbA1C 5.7-6.4% as Prediabetes, HbA1C >6.5% as Diabetes [16].

## 2. MATERIALS AND METHODS

### 2.1 Materials

Materials were as follows: Candesartan cilexetil (Atacand®) was obtained from local market and manufactured by AstraZeneca pharmaceutical company (Sweden) as tablet form 16 mg/tablet. Used as anti-hypertensive agent. Enalapril Maleate (Korandil®) was obtained from local market and manufactured by Remedica Ltd pharmaceutical company (Cyprus, Europe) as tablet form 10 mg/tablet used as anti-hypertensive agent. Glimepiride (Amaryl®) was obtained from local market and manufactured by Sanofi pharmaceutical company (France) as tablet form 4 mg/tablet used for DM type-2 treatment (Table 1). Streptozocin (75% α-anomer basis powder) purchased from local market and manufactured by Sigma Aldrich which is known in Merck Germany (B.N. S6130SA) was used to induce type-2 DM for medical research.

### 2.2 Instrumentation

Instrument was as follows: The i-CHROMA ™ reader which is a first-generation portable fluorescent scanner that analyzes blood, urine, and other samples and displays measurement results on the screen. More than 30 immunoassay test cartridges can be measured. Furthermore, i-CHROMA™ Reader HbA1c is an immunoassay system for quantitative measurement of hemoglobin A1c in Human blood with i-CHROMA™ Reader. The test is used for routine monitoring of the long-term glycemic status in patients with DM.

Incubator (i-Chamber) is Incubation chamber for testing HbA1C.

Cartridge (kits) for measuring concentration of rat's hemoglobin A1c (HbA1c) in biological liquid samples (Accu-Chek products).

### 2.3 Reagent

Reagent was as follows:

- Sodium Citrate buffer used to dissolve Streptozocin.
- Hemolysis Buffer is pre-dispensed individually in a small tube and composed of cationic detergent, is stable up to 20 months if stored at 4 - 30°C in its sealed pouch.
- Detection Buffer contains fluorescence-labeled HbA1c-peptide, fluorescence-labeled anti-rabbit IgG, BSA as a stabilizer and sodium azide as preservative in PBS, is stable up to 20 months, if stored at 2 - 8°C, allow detection buffer to reach room temperature (20-30°C) before starting a test.
- Distilled water.

### 2.4 Methods

Preparation of Streptozocin solution: Streptozocin at dose 45 mg, was prepared by dissolving 45 mg of Streptozocin in 12 ml of Sodium Citrate Buffer, and mixed in a forced blender/mixer to get homogenous solution of 3.75%(w/v) concentration of Streptozocin, which

give (3.75 mg/1ml) conc. of solution injected IP (Intraperitoneal) for each rat [17,18].

Preparation of Candesartan cilexetil solution: Candesartan cilexetil at dose 16 mg (16 mg/70 kg), (0.228 mg/1 kg), (0.057 mg/0.25 kg) was prepared daily, by dissolving 16 mg of Candesartan cilexetil in 200 ml of distilled water and mixed in a forced blender/mixer to get homogenous solution of 0.08%(w/v), concentration of Candesartan cilexetil, which provides 0.057 mg/0.8 ml of solution orally for each rat [19].

Preparation of Enalapril Maleate solution: Enalapril Maleate at dose 10 mg (10 mg/70 kg), (0.142 mg/1 kg), (0.0357 mg/0.25 kg) was prepared daily, by dissolving 10 mg of Enalapril Maleate in 100 ml of distilled water and mixed in a forced blender/mixer to get homogeneous solution of 0.1%(w/v) concentration of Enalapril Maleate, which provides 0.0357/0.4 ml of solution orally for each rat [20].

Preparation of Glimepiride solution. Glimepiride at dose 4 mg (4 mg/70 kg), (0.0571 mg/1 kg), (0.0142 mg/0.25 kg) was prepared daily, by dissolving 4 mg of Glimepiride in 100 ml of distilled water and mixed in a forced blender/mixer to get homogeneous solution of 0.04%(w/v) concentration of Glimepiride, which provides 0.0142 mg/ 0.3 ml of solution orally for each rat [11].

Preparation of sodium citrate buffer solution. Sodium citrate buffer solution was prepared by adding 800 ml distilled water in a container, followed by addition of add 24.0 g of sodium citrate dehydrate, add 3.5 g of citric acid to the solution. pH of solution was adjusted to 4.5 pH, and storage stored at in 4-10°C.

## 2.5 Animal Handling (Fig. 5)

Wistar albino adult male and female laboratory rats (Ethical Committee approval - Appendix 1) above 8 weeks, a and weigh between 250-300 gm were supplied by animal house of the Applied Science Private University, and were placed in air-conditioned room at 20-25°C and exposed to photoperiod cycle (12 hours light / 12 hours dark) daily. DM type 2 was induced in all rats by STZ injection for consecutive five days. Two weeks later all rats were marked on their tails for the identification, weighed and randomized into four groups.

- Group #I: Diabetic 25 rats which were not given any drugs, and fedon normal basal diet with water throughout the 12 weeks of the study period under the same environmental conditions.
- Group #II: Diabetic 25 rats which were given Glimepiride only and fed on normal basal diet with water.
- Group #III: Diabetic 25 rats which were given Glimepiride and Candesartan cilexetil and fed on normal basal diet with water.
- Group #IV: Diabetic 25 rats which were given Glimepiride and Enalapril and fed on normal basal diet with water.

Serum blood samples were drawn through the optical vein of rat by capillary tube after 12 hours of fasting before sample collecting. The sample collected at zero time to determine the baseline and then collected at two week and twelve weeks of the randomization process. When samples were drawn from rats and put in tube collector (with E DTA, heparin and NaF). HbA1c level was measured by placing blood samples in Cartridges (kit- Special for rats), and put in an incubator chamber for 12 minutes and finally placed in the i-Chroma™ reader to read the HbA1c range.

Statistical Analysis: The Statistical Package for the Social Sciences Software (SPSS Version 22, IBM 2016) was utilized for performing data analysis. Descriptive statistics such as percentages, means, standard deviations, etc., were used to describe the HbA1c levels of the study animals. This was followed by analyzing the data using the appropriate inferential statistical tests such as Analysis of Variance (ANOVA), and repeated measures ANOVA.

## 3. RESULTS

HbA1C mean levels of the four study groups were measured at baseline, two-weeks, and 12 weeks' period (Table 2). For instance; the mean +SD HbA1C of group I at zero-time (pre-test) was = 7.62 +SD 0.41 which increased to 7.71 +SD 0.44 two weeks' post-test, and then decreased again to 7.62 +SD 0.41 at 12 weeks' post-test (Table 2).

There were no significant differences (p-values <0.05) between the four groups (groups I, II, III,

and IV) in the HbA1C mean level at the beginning (bassline) of this study (Table 3). For instance; the mean difference between the Glimepiride group and Glimepiride + Candesartan group was 0.06 with p-value of 0.97 (>0.05).

Two weeks later, treated groups (groups II, III, and IV) showed moderate reduction of HbA1C mean level compared to the untreated (placebo) group I, that was significant in groups III, and IV, and insignificant in group II (mean = 7.43 ±SD 0.54, 6.97 ±SD 0.33, 6.72 ±SD 0.26, and 7.71 ±SD 0.44 respectively) (Table 4).

Furthermore, treated groups (groups II, III, and IV) showed significant dramatic reduction of HbA1C mean level when compared to the untreated group (group I) (mean = 6.22 +SD 0.51, 5.24 +SD 0.62, 5.22 +SD 0.13, and 7.62 +SD 0.42 respectively (Table 5).

Overall, treated groups showed significantly lower HbA1C level than placebo groups. Moreover, Glimepiride + Enalapril combination showed a stronger hypoglycemic effect than the Glimepiride + Candesartan combination at post, and post-delayed tests, however, these differences were not significant (Table 6, and Fig. 4).

## 4. DISCUSSION

Our study illustrates that the antihypertensive medications such as ACE inhibitors and ARB's significantly reduced HbA1C level in NIDDM rats, when given in combinations with other anti-diabetic drugs like glimepiride and this goes in congruence with earlier reports [14,15]. Hypertension-related increase of intracellular free calcium results into cellular resistance to insulin, However, insulin responsiveness was back to normal in the presence of antihypertensive medications like nifedipine and verapamil [20-31]. Limula et al. [28] concluded that ACE inhibitors like Enalapril and ARB's like Candesartan enhance insulin sensitivity and prevent resistance in rats fed on fructose-and in high blood pressure patients. Furthermore, it was reported that improvement in insulin sensitivity of ACE inhibitors and ARB's may be due to inhibition of the signaling of angiotensin-II. Moreover, ACE inhibitors and ARB's boost the insulin responsiveness for effective glucose elimination. A clinical study reported that ACE inhibitors and ARB's blocks vasoconstriction induced by Ang-II induced and therefore improves glucose transport to skeletal muscle through improving insulin sensitivity [19]. Either clinical or experimental studies report that ACE inhibition improves not only skeletal muscle glucose uptake stimulated by insulin, but also the blood flow in insulin resistant states through directly stimulating the cellular glucose uptake [29,30]. In conclusion, our study adds to the literature a piece of evidence that antihypertensive drugs might interfere with blood sugar level in diabetic patients, especially in patients who are on antidiabetic medications and thus this interaction should be considered by healthcare professionals who are attending patients who are taking both drug classes together(Fig. 4).

**Table 2. Mean ± SD of control and intervention groups at pre, post, and post-delayed HbA1c readings**

| Treatment | | Mean | Std. deviation | N |
|---|---|---|---|---|
| **Pre-test** | Placebo | 7.62 | 0.41 | 25 |
| | Glimepiride | 7.72 | 0.48 | 25 |
| | Glimepiride + Candesartan | 7.66 | 0.47 | 25 |
| | Glimepiride + Enalapril | 7.52 | 0.51 | 25 |
| **Post-test (2 weeks)** | Placebo | 7.71 | 0.44 | 25 |
| | Glimepiride | 7.43 | 0.54 | 25 |
| | Glimepiride + Candesartan | 6.97 | 0.33 | 25 |
| | Glimepiride + Enalapril | 6.72 | 0.26 | 25 |
| **Post-delayed (12 weeks)** | Placebo | 7.62 | 0.42 | 25 |
| | Glimepiride | 6.22 | 0.51 | 25 |
| | Glimepiride + Candesartan | 5.24 | 0.62 | 25 |
| | Glimepiride + Enalapril | 5.22 | 0.13 | 25 |

**Table 3. The differences between HbA1C levels of the four study groups at baseline**

| Dependent variable | (I) Treatment | (J) Treatment | Mean difference (I-J) | Std. error | Sig. | 95% confidence interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower bound | Upper bound |
| **Pre _ test** | **Placebo** | Glimepiride | -0.09600 | 0.13241 | 0.887 | -0.44 | 0.25 |
| | | Glimepiride + Candesartan | -0.04 | 0.13241 | 0.99 | -0.38 | 0.31 |
| | | Glimepiride + Enalapril | 0.11 | 0.13241 | 0.85 | -0.24 | 0.45 |
| | **Glimepiride** | Placebo | 0.07 | 0.13241 | 0.89 | -0.25 | 0.44 |
| | | Glimepiride + Candesartan | 0.06 | 0.13241 | 0.97 | -0.29 | 0.41 |
| | | Glimepiride + Enalapril | 0.20 | 0.13241 | 0.42 | -0.14 | 0.55 |
| | **Glimepiride + Candesartan** | Placebo | 0.04 | 0.13241 | 0.99 | -0.31 | 0.38 |
| | | Glimepiride | -0.06 | 0.13241 | 0.97 | -0.41 | 0.29 |
| | | Glimepiride + Enalapril | 0.14 | 0.13241 | 0.70 | -0.20 | 0.49 |
| | **Glimepiride + Enalapril** | Placebo | -0.11 | 0.13241 | 0.85 | -0.45 | 0.24 |
| | | Glimepiride + Candesartan | -0.20 | 0.13241 | 0.42 | -0.55 | 0.14 |
| | | Glimepiride + Enalapril | -0.14 | 0.13241 | 0.70 | -0.49 | 0.20 |

**The mean difference is significant at the 0.05 level*

**Table 4. The differences between HbA1C levels of the four study groups at two-weeks**

| Dependent variable | (I) Treatment | (J) Treatment | Mean difference (I-J) | Std. error | Sig. | 95% confidence interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower bound | Upper bound |
| **Post _ test** | **Placebo** | Glimepiride | 0.28400 | 0.11508 | 0.072 | -0.0169 | 0.5849 |
| | | Glimepiride + Candesartan | 0.74400* | 0.11508 | 0.000 | 0.4431 | 1.0449 |
| | | Glimepiride + Enalapril | 0.99200* | 0.11508 | 0.000 | 0.6911 | 1.2929 |
| | **Glimepiride** | Placebo | -0.28400 | 0.11508 | 0.072 | -0.5849 | 0.0169 |
| | | Glimepiride + Candesartan | 0.46000* | 0.11508 | 0.001 | 0.1591 | 0.7609 |
| | | Glimepiride + Enalapril | 0.70800* | 0.11508 | 0.000 | 0.4071 | 1.0089 |
| | **Glimepiride + Candesartan** | Placebo | -0.74400* | 0.11508 | 0.000 | -1.0449 | -0.4431 |
| | | Glimepiride | -0.46000* | 0.11508 | 0.001 | -0.7609 | -0.1591 |
| | | Glimepiride + Enalapril | 0.24800 | 0.11508 | 0.143 | -0.0529 | 0.5489 |
| | **Glimepiride + Enalapril** | Placebo | -0.99200* | 0.11508 | 0.000 | -1.2929 | -0.6911 |
| | | Glimepiride | -0.70800* | 0.11508 | 0.000 | -1.0089 | -0.4071 |
| | | Glimepiride + Candesartan | -0.24800 | 0.11508 | 0.143 | -0.5489 | 0.0529 |

**The mean difference is significant at the 0.05 level*

**Table 5. The differences between HbA1C levels of the four study groups at 12-weeks**

| Dependent variable | (I) Treatment | (J) Treatment | Mean difference (I-J) | Std. error | Sig. | 95% confidence interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower bound | Upper bound |
| **Post _ delayed** | **Placebo** | Glimepiride | 1.40000* | 0.12916 | 0.000 | 1.0623 | 1.7377 |
| | | Glimepiride + Candesartan | 2.38400* | 0.12916 | 0.000 | 2.0463 | 2.7217 |
| | | Glimepiride + Enalapril | 2.40000* | 0.12916 | 0.000 | 2.0623 | 2.7377 |
| | **Glimepiride** | Placebo | -1.40000* | 0.12916 | 0.000 | -1.7377 | -1.0623 |
| | | Glimepiride + Candesartan | 0.98400* | 0.12916 | 0.000 | 0.6463 | 1.3217 |
| | | Glimepiride + Enalapril | 1.00000* | 0.12916 | 0.000 | 0.6623 | 1.3377 |
| | **Glimepiride + Candesartan** | Placebo | -2.38400* | 0.12916 | 0.000 | -2.7217 | -2.0463 |
| | | Glimepiride | -0.98400* | 0.12916 | 0.000 | -1.3217 | -0.6463 |
| | | Glimepiride + Enalapril | 0.01600 | 0.12916 | 0.999 | -0.3217 | 0.3537 |
| | **Glimepiride + Enalapril** | Placebo | -2.40000* | 0.12916 | 0.000 | -2.7377 | -2.0623 |
| | | Glimepiride | -1.00000* | 0.12916 | 0.000 | -1.3377 | -0.6623 |
| | | Glimepiride + Candesartan | -0.01600 | 0.12916 | 0.999 | -0.3537 | 0.3217 |

**The mean difference is significant at the 0.05 level*

**Table 6. The overall differences between HbA1C levels of the four study groups throughout the study period (12 weeks)**

| Treatment * Time | | | | | |
|---|---|---|---|---|---|
| **Treatment** | **Mean difference (I-J)** | **Std. error** | **Sig.** | **95% confidence interval** | |
| | | | | **Lower bound** | **Upper bound** |
| **Placebo** | 0.5293* | 0.06925 | 0.000 | 0.3428 | 0.7159 |
| | 1.0307* | 0.06925 | 0.000 | 0.8441 | 1.2172 |
| | 1.1667* | 0.06925 | 0.000 | 0.9801 | 1.3532 |
| **Glimepiride** | -0.5293* | 0.06925 | 0.000 | -0.7159 | -0.3428 |
| | 0.5013* | 0.06925 | 0.000 | 0.3148 | 0.6879 |
| | 0.6373* | 0.06925 | 0.000 | 0.4508 | 0.8239 |
| **Glimepiride + Candesartan** | -1.0307* | 0.06925 | 0.000 | -1.2172 | -0.8441 |
| | -0.5013* | 0.06925 | 0.000 | -0.6879 | -0.3148 |
| | 0.1360 | 0.06925 | 0.315 | -0.0506 | 0.3226 |
| **Glimepiride + Enalapril** | -1.1667* | 0.06925 | 0.000 | -1.3532 | -0.9801 |
| | -0.6373* | 0.06925 | 0.000 | -0.8239 | -0.4508 |
| | -0.1360 | 0.06925 | 0.315 | -0.3226 | 0.0506 |

*Based on observed means; The error term is Mean Square (Error) = 0.060; *. The mean difference is significant at the0.05 level*

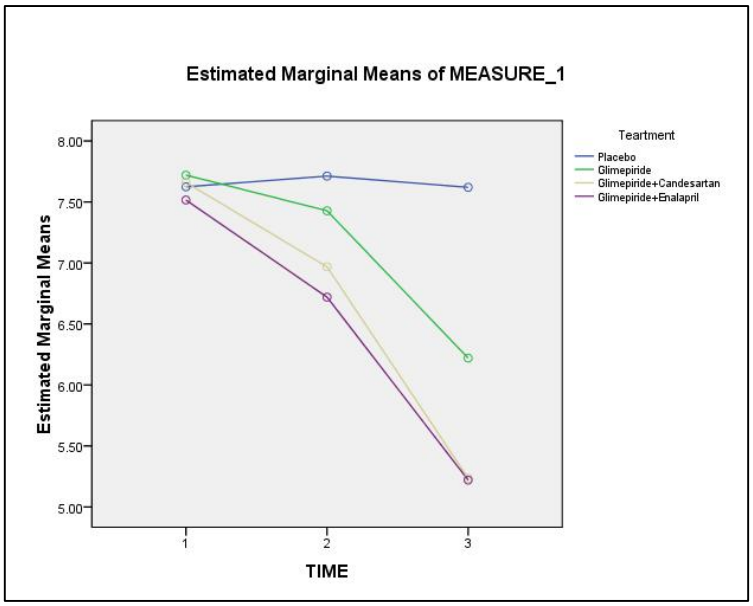


**Fig. 4. The change in HbA1C level in the four groups over the three time points (0, 2, and 12 weeks)**

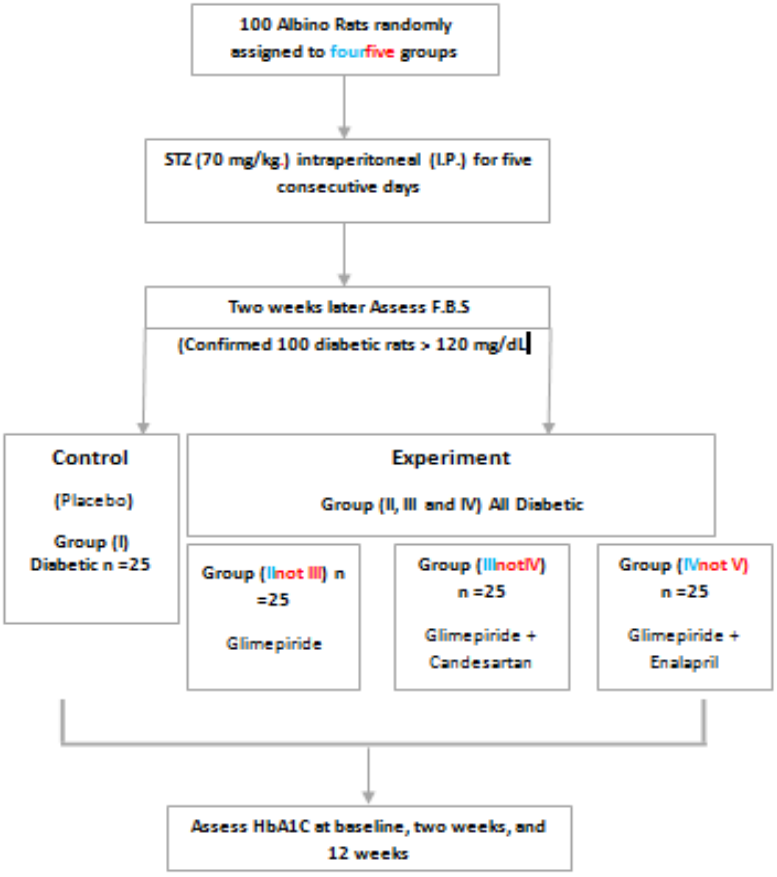


**Fig. 5. Flowchart of the study**

## 5. CONCLUSION

The close relationship between DM and HTN has been recognized for decades, however, the interactions between the drugs of the two conditions are still controversial with conflicting evidences. Our study highlighted the effects of antihypertensive agents (ACE-inhibitor: Enalapril, and ARB: Candesartan) on STZ-induced type2 diabetic rates, when given in combination with anti-diabetic drug from the sulfonylurea group; glimepiride. A short-term moderate, and long - term dramatic reduction in HbA1c (Glycated hemoglobin) level were recorded in rats' groups treated with glimepiride + Candesartan, or glimepiride + Enalapril combinations compared to their counterparts treated with placebo or Glimepiride alone. The reduction in HbA1C level was higher in Enalapril combinations compared to Candesartan ones, however, this reduction was not significant. A close monitoring and dose-adjustment procedures should be performed in patients who are newly adding an ARB or ACEI to their DM regimen to avoid the risk of hyperglycemia in patients suffering from both HTN and DM type 2.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s). The approval number granted is "AAU-Pharm-9/2018.

## ACKNOWLEDGEMENT

The authors would like to thank the Faculty of Pharmacy and the Medical Sciences University of Petra, also the team like to extend their thanks to the technicians of the animal house at ASS Mr. Salem and all who collaborated for their help in offering me the resources in running the research.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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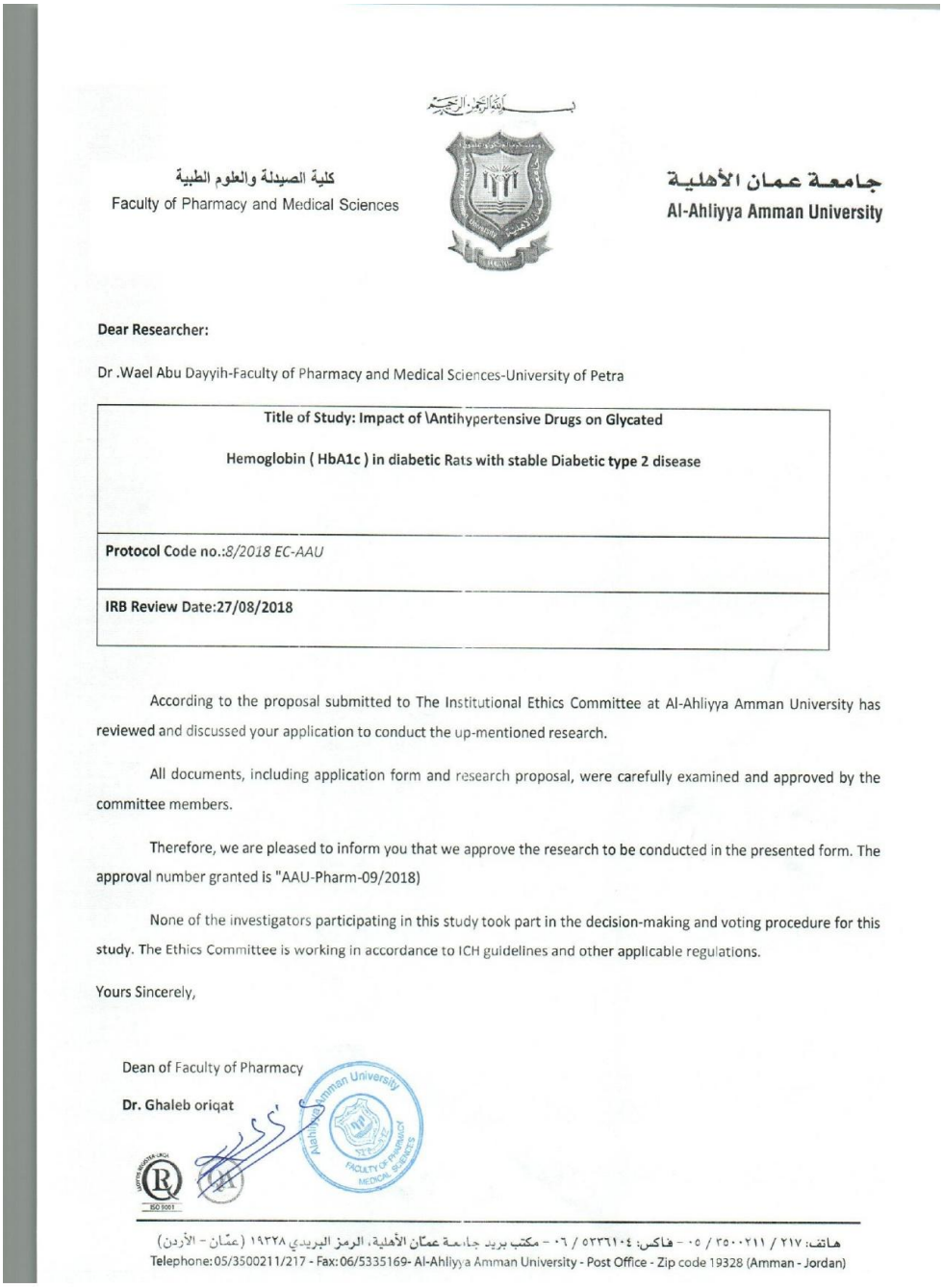
## APPENDIX 1. ETHICAL COMMITTEE APPROVAL



بسم الله الرحمن الرحيم

كلية الصيدلة والعلوم الطبية
Faculty of Pharmacy and Medical Sciences

جامعة عمان الأهلية
Al-Ahliyya Amman University

**Dear Researcher:**

Dr .Wael Abu Dayyih-Faculty of Pharmacy and Medical Sciences-University of Petra

| **Title of Study: Impact of \Antihypertensive Drugs on Glycated Hemoglobin ( HbA1c ) in diabetic Rats with stable Diabetic type 2 disease** |
|---|
| **Protocol Code no.:***8/2018 EC-AAU* |
| **IRB Review Date:27/08/2018** |

According to the proposal submitted to The Institutional Ethics Committee at Al-Ahliyya Amman University has reviewed and discussed your application to conduct the up-mentioned research.

All documents, including application form and research proposal, were carefully examined and approved by the committee members.

Therefore, we are pleased to inform you that we approve the research to be conducted in the presented form. The approval number granted is "AAU-Pharm-09/2018)

None of the investigators participating in this study took part in the decision-making and voting procedure for this study. The Ethics Committee is working in accordance to ICH guidelines and other applicable regulations.

Yours Sincerely,

Dean of Faculty of Pharmacy

**Dr. Ghaleb oriqat**

هاتف: ٢١٧ / ٣٥٠٠٢١١ / ٠٥ - فاكس: ٥٣٣٦١٠٤ / ٠٦ - مكتب بريد جامعة عمّان الأهلية، الرمز البريدي ١٩٣٢٨ (عمّان - الأردن)
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